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COMPARATIVE TOXICITY AND TOXICOKINETICS OF DDT AND ITS MAJOR METABOLITES IN FRESHWATER AMPHIPODS

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Abstract—The toxicity and toxicokinetics of radiolabeled DDT and its major degradation products, dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), were determined for the amphipods *Hyalella azteca* and *Diporeia* spp. in water-only static renewal exposures. Comparison of the water and tissue concentrations associated with decreased survival revealed large differences in toxicity among the three compounds. In *H. azteca,* the ratio of the 10-d LR50 values (median lethal tissue residue) for DDT:DDD:DDE was 1:24:195. In *Diporeia* spp., the 28-d LR50 for DDT was higher than that for DDD by a factor of six, and DDE did not cause significant mortality even at concentrations approaching the solubility limit. Based on the toxicity data, the hazard from exposure to mixtures of DDT and its degradation products should be evaluated on a toxic-units basis and not as a simple summation of the individual concentrations, which ignores the toxicity of specific compounds. Differences in species sensitivity were also detected. The 10-d LR50 values were higher in *Diporeia* spp. than in *H. azteca* by a factor of 40 for DDT and eight for DDD. This difference can be only partly attributed to differences in lipid content between *H. azteca* (7% dry wt) and *Diporeia* spp. (24% dry wt). The uptake clearance and elimination rate constants were similar among the various compounds in both species. Uptake clearance was typically fourfold greater for *H. azteca* than for *Diporeia* spp., however, and the experimentally measured elimination rate was approximately 30-fold greater in *H. azteca* than in *Diporeia* spp. The larger rates of uptake and elimination were attributed to the higher exposure temperature, greater surface area-to-volume ratio, and lower lipid content for *H. azteca* compared with *Diporeia* spp. In addition, extensive biotransformation of DDT by *H. azteca* may have contributed to a more rapid compound elimination.

Keywords—DDT Dichlorodiphenyldichloroethane Dichlorodiphenyldichloroethylene Amphipods Critical body residue

INTRODUCTION

One of the best-known and most studied chlorinated hydrocarbon contaminants is DDT. Despite a ban on its manufacture in the United States imposed during the early 1970s, DDT is still present in the environment and in the biota [1–6]. The lipophilic character of DDT facilitates its accumulation and persistence in lipid-rich tissues of the biota, and its biomagnification up the food chain is a major concern [7]. Frequently, DDT co-occurs with its breakdown products, mostly dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). Concentrations of DDD and DDE may exceed that of the parent compound (DDT) in highly contaminated sites [1–4,8]. In addition, DDD has been manufactured as a pesticide and released into the environment. Even so, DDE is considered to be more persistent than either DDT or DDD [7].

Dichlorodiphenyltrichloroethane, DDD, and DDE are toxic to aquatic invertebrates. Comparative studies suggest that their relative acute toxicities differ greatly and are species specific [3,4,9,10]. In those studies, the relative toxicity of the different compounds to a single species or the toxicity of a single compound to different species was established using point estimates (LC50) derived from water exposures with fixed durations (e.g., 96 h, 10 d) [3,4,9,10]. This approach assumes, however, that the relationship between environmental concentration and manifestation of a biologic effect is the same among organisms or compounds. Because biologic effects are manifestations of contaminants within the organisms, solely determining the environmental concentration associated with toxicity to establish comparisons is likely to be inaccurate. Even under constant conditions, the pattern of bioaccumulation is different for a given species exposed to different compounds or among different species exposed to the same compound. Accurate assessment of the relationship between environmental concentrations and the manifestation of toxicity is only possible when toxicokinetic parameters are measured. Similarly, assessment of species sensitivity is more accurate when observed biologic effects are related to the concentration of a contaminant at the site of action (e.g., tissues) [11].

Despite differences in their chemical traits (e.g., hydrophobicity, water solubility) and apparent differences in their toxicities to benthic invertebrates, DDT, DDD, and DDE have been treated as a single toxicologic entity in numerous studies. In the sum DDT (ΣDDT) approach, the individual concentrations of the three compounds are combined, so the individual contribution of each congener is not considered [6,8,12,13]. Comparative studies, especially those incorporating body res-

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Table 1. *Hyalella azteca* toxicity experiments with dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyltrichloroethylene (DDE)[a]

Compound	Water concentration (μg/L)	Water concentration % decline	% Survival day 10	k_u (ml/g/h)	k_e (h^{-1})	$k_{e(em)}$ (h^{-1})	$t_{1/2}$ (d)	BCFss ($k_u/k_{e(m)}$)
DDT	0.024 (0.002)	38.5 (15.7)	98 (8)	269.0 (42.0)	0.0047 (0.0018)	0.0099 (0.0010)	2.9	27,172
	0.043 (0.004)	25.7 (16.7)	80 (5)	316.5 (73.0)	0.0082 (0.0030)	0.0082 (0.0012)	3.5	38,598
	0.064 (0.006)	25.3 (14.9)	86 (9)	244.6 (47.9)	0.0064 (0.0025)	0.0102 (0.0009)	2.8	23,980
	0.122 (0.018)	26.6 (13.1)	36 (9)[b]	293.8 (123.0)	0.0137 (0.0077)	0.0074 (0.0016)	3.9	39,703
	0.211 (0.018)	32.6 (14.0)	0[b]	212.9 (118.0)	0.0091 (0.0130)	ND		
	0.358 (0.030)	34.7 (18.1)	0[b]	222.3 (654.0)	0.0270 (0.1660)	ND		
DDD	0.095 (0.015)	32.7 (9.7)	96 (5)	367.15 (62.1)	0.0075 (0.0023)	ND		
	0.178 (0.021)	35.1 (8.3)	91 (6)	334.4 (108.0)	0.0133 (0.0057)	0.0200 (0.0024)	1.5	16,720
	0.366 (0.070)	35.1 (11.3)	96 (8)	272.9 (65.5)	0.0084 (0.0033)	ND		
	0.692 (0.158)	39.6 (8.0)	61 (19)[b]	305.3 (82.5)	0.0126 (0.0046)	ND		
	1.381 (0.158)	40.6 (7.7)	8 (3)[b]	204.2 (33.6)	0.0031 (0.0016)	ND		
DDE	1.117 (0.082)	56.5 (9.9)	94 (5)	543.1 (111.6)	0.0161 (0.0040)	0.0123 (0.0007)	2.3	44,154
	2.258 (0.186)	60.1 (11.7)	85 (8)[b]	653.3 (230.8)	0.0213 (0.0087)	ND		
	4.947 (0.542)	62.0 (4.0)	60 (13)[b]	491.1 (220.0)	0.0220 (0.0113)	ND		
	8.208 (1.533)	56.3 (4.0)	0[b]	345.6 (62.7)	0.0054 (0.0036)	ND		
	22.021 (0.263)	37.1 (16.8)	0[b]	169.4 (36.6)	0	ND		

[a] Mean (standard deviation) water concentration, percentage decline in water concentration between daily exchange, and mean percentage (%) survival at day 10, best estimate from nonlinear regression (standard error) for uptake clearance rate (k_u), elimination rate (k_e), and experimentally measured elimination rate ($k_{e(m)}$), and the elimination half-life ($t_{1/2}$) and steady-state bioconcentration factors (BCFss). ND = not determined.
[b] Significant difference from the control ($p < 0.05$).

idue measurements and toxicokinetics, are necessary for deriving more appropriate procedures to assess the hazards associated with exposure to mixtures of DDT and its metabolites.

In this study, the toxicities of radiolabeled DDT, DDD, and DDE were compared and interspecific differences in sensitivity assessed using the freshwater amphipods *Hyalella azteca* and *Diporeia* spp. A time series of water exposures to the three compounds allowed the calculation of toxicity point estimates derived from environmental concentrations (median lethal concentration [LC50]) and from body residues (median lethal tissue residue [LR50]) and toxicokinetic parameters (e.g., uptake clearance rate, elimination rate, steady-state bioconcentration factor). Because DDT can be enzymatically metabolized to DDD or DDE, the impact of biotransformation on toxicity was also evaluated. Toxicity and bioaccumulation data were integrated to allow accurate comparisons among compounds and between species.

MATERIAL AND METHODS

Chemicals

The chemicals [^{14}C]*p,p'*-DDT (18.7 mCi/mmol), [^{14}C]*p,p'*-DDD (2.6 mCi/mmol), and [^{14}C]*p,p'*-DDE (13.4 mCi/mmol) were purchased from Sigma Chemical (St. Louis, MO, USA). Molecular weights for DDT, DDD, and DDE are 354.48, 320.1 and 318.03, respectively. Compounds were tested for radiopurity before use by thin-layer chromatography (hexane:benzene, 95:5, v:v) on silica plates. All compounds were determined to >98% pure. Dosing stocks of radiolabeled compounds were prepared by diluting an aliquot of the original stock with the appropriate volume of acetone.

Exposure media

Water used in all experiments was collected from the Huron River upstream from Dexter, MI, USA, at the Hudson Mills Metropark. Hardness (165–250, $n = 15$), alkalinity (170–250, $n = 15$), and pH (8.1–8.3, $n = 15$) were measured before use in each experiment, where n is the total number of measurements before each experiment. Test solutions were prepared by adding a known amount of [^{14}C]DDT, [^{14}C]DDD, or [^{14}C]DDE working stock to 3 L of filtered water (glass microfiber filters 934-AH; Whatman, Clifton, NJ, USA). The volume of carrier solvent in the water never exceeded 60 μl/L.

Samples (2 ml) of spiked water were taken to determine the amount of chemical bound to dissolved organic matter by determining the [^{14}C] activity in a sample before and after passing through a C_{18} reversed-phase cartridge [14]. The amount of compound passing through the cartridge was assumed to be the amount of compound bound to the dissolved organic matter.

Organisms

Laboratory-reared *H. azteca* that passed through a 0.5-mm mesh and were retained in a 0.355-mm mesh (juvenile organisms ≈1–2 weeks old) were used in all experiments. Field-collected *Diporeia* spp. [15] that passed through a 2-mm mesh and were retained in a 1-mm mesh (juvenile organisms ≈5–11 months old) were used in all experiments.

Toxicity and bioaccumulation experiments

Toxicity and bioaccumulation of [^{14}C]DDT, [^{14}C]DDD, and [^{14}C]DDE were examined in 10-d (*H. azteca*) or 28-d (*Diporeia* spp.), water-only exposures. Amphipods were exposed to a range of contaminant concentrations (Tables 1 and 2) in 300-ml beakers filled with 250 ml of test solution. Each exposure beaker received 20 (*H. azteca*) or 10 (*Diporeia* spp.) test organisms at day 0. A 1-cm square of sterile cotton surgical gauze was placed in the beakers with *H. azteca* for substrate, and 0.5 ml of yeast-cerophyl-trout-chow (YCT) was added every second day [16]. Beakers with *Diporeia* spp. received no substrate or food. *Diporeia* spp. can undergo long periods of starvation (1–2 months) with little decline in survival and lipid content [17]. Experiments with *Diporeia* spp. were conducted at 4°C and with *H. azteca* at room temperature (18.2–

Table 2. *Diporeia* spp. toxicity experiments with dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE)[a]

Compound	Water concentration (μg/L)	Water concentration % decline	% Survival day 28	k_u (ml/g/h)	k_e (h^{-1})	$k_{e(em)}$ (h^{-1})	$t_{1/2}$ (d)	BCFss ($k_u/k_{e(m)}$)
DDT	0.221 (0.048)	38.2 (10.2)	80 (14)	65.2 (3.8)	0.0007 (0.00003)	0.0003 (0.0001)	96.3	217,333
	0.352 (0.066)	43.3 (9.3)	71 (10)[b]	70.3 (9.8)	0.0010 (0.0004)	ND		
	0.826 (0.133)	32.8 (15.9)	8 (5)[b]	74.3 (6.2)	0.0010 (0.0003)	ND		
	1.595 (0.344)	30.5 (17.2)	0[b]	82.3 (16.8)	0.0027 (0.0014)	ND		
	3.319 (0.726)	33.0 (13.9)	0[b]	64.3 (13.0)	0.0027 (0.0020)	0.0002 (0.0001)	144.4	321,500
DDD	0.944 (0.174)	34.3 (18.9)	83 (8)[b]	87.2 (24.3)	0.0005 (0.0009)	0.0002 (0.0001)	144.4	436,000
	2.791 (0.790)	30.2 (13.2)	32 (19)[b]	79.0 (34.0)	0.0008 (0.0016)	ND		
	7.420 (1.346)	26.8 (15.7)	0[b]	142.8 (50.0)	0.0116 (0.0050)	ND		
	17.050 (3.430)	29.34 (10.6)	0[b]	114.1 (17.8)	0.0103 (0.0025)	ND		
DDE	2.293 (0.646)	46.5 (19.3)	93 (15)	102.2 (24.2)	0.0006 (0.0008)	0.0002 (0.0001)	144.4	511,000
	4.726 (1.082)	46.3 (19.4)	81 (26)	93.5 (10.2)	0.0005 (0.0004)	ND		
	9.141 (1.494)	45.3 (17.8)	84 (13)	78.0 (9.4)	0.0009 (0.0004)	ND		
	20.194 (4.971)	41.0 (14.7)	90 (14)	61.5 (8.7)	0.0023 (0.0006)	0.0002 (0.0001)	144.4	307,500

[a] Mean (standard deviation) water concentration, percentage decline in water concentration between daily exchange, and mean percentage (%) survival at day 28, best estimate from nonlinear regression (standard error) for uptake clearance rate (k_u), elimination rate (k_e), and experimentally measured elimination rate ($k_{e(m)}$), and the elimination half-life ($t_{1/2}$) and steady-state bioconcentration factors (BCFss). ND = not determined.
[b] Significant difference from the control ($p < 0.05$).

21.0°C). Experiments with *H. azteca* were initiated on January 1, 1997, for DDT, on April 1, 1997, for DDD, and on May 12, 1997, for DDE. All experiments with *Diporeia* spp. were initiated on July 21, 1997, and shared the same set of control beakers.

For *H. azteca* and *Diporeia* spp., four beakers (five in the DDT *H. azteca* experiment) per treatment were used for monitoring survival. Each day for the duration of the experiment, the number of live amphipods in each exposure beaker was recorded, and dead (completely immobilized) amphipods were removed. In addition, the number of *Diporeia* spp. that appeared to be narcotized was also recorded at days 10 and 28. *Narcosis* is defined as the inability of an amphipod to actively swim on contact stimulus. When the experiment was completed, surviving amphipods were used for body residue or lipid content determinations. Extra replicate beakers were included in the experimental design to sample amphipods for body residue at intermediate time points. In the *H. azteca* experiments, beakers were sampled at days 1, 2, 4, and 6; two beakers (three in the DDT experiment) were sampled per sampling period. In the *Diporeia* spp. experiments, amphipods were sampled from the same set of two beakers per treatment at days 2 and 5 and from a different set of two beakers at days 10 and 17.

Three-fourths of the test-solution in each beaker was exchanged daily. Five milliliters of exposure water were sampled in triplicate for compound concentration at the beginning of the experiment (day 0) and then daily from each treatment. Amphipods from the exposure beakers were sampled in groups of two to five individuals at the end of the experiment and from extra beakers at intermediate time points. These amphipods were blotted dry and weighed. Water and amphipod samples were transferred to a 12-ml scintillation cocktail (3a70b; Research Products International, IL, USA), and [^{14}C] activity was quantified by liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR; Packard Instruments, Meridien, CT, USA). Specific activities were used for converting the radioactivity concentration in water or amphipod samples to the molar concentration of contaminants. Because compound concentrations were calculated using [^{14}C] activity as a surrogate, all concentrations are reported as parent compound equivalents, which included parent compound and breakdown products. The relative proportion of parent compound and breakdown products in *Diporeia* spp. was determined at the end of the 28-d exposure and in *H. azteca* in separate experiments. Negligible transformation of DDT, DDD, and DDE was expected in the water during the exposures, because transformation was not observed in similar experiments reported elsewhere [3].

Lipid content of *H. azteca* and *Diporeia* spp. was determined at day 0 and at the end of the experiment using the organisms sampled from the control group and from one selected treatment (*H. azteca*: 0.122 μg/L DDT, 0.692 μg/L DDD, and 8.208 μg/L DDE; *Diporeia* spp.: 0.352 μg/L DDT, 0.944 μg/L DDD, and 20.194 μg/L DDE). The percentage of total lipids was determined using a microgravimetric method [18].

Elimination experiments

Hyallela azteca (n = 135 per dose) were exposed to the four lowest concentrations of [^{14}C]DDT used in the toxicity experiment (Table 1) in 1-L beakers filled with 800 ml of spiked Huron River water. After a 48-h static exposure, amphipods were transferred to uncontaminated water in three 600-ml beakers. Three-fourths of the water was exchanged daily to prevent reabsorption of the eliminated compound, and amphipods were fed 2 ml of YCT every second day. Groups of seven to 12 individuals were retrieved from each beaker after 0, 3, 5, and 8 d; these amphipods were then weighed and assayed for radioactivity using LSC. In addition, *H. azteca* (~135 per compound) were exposed to the lowest concentration of [^{14}C]DDD and [^{14}C]DDE used in the toxicity experiment (Table 1), transferred to uncontaminated water, and then sampled after 0, 2, 4, and 6 d as described for [^{14}C]DDT.

Diporeia spp. (n = 30 per dose) were exposed to the lowest and highest concentrations of [^{14}C]DDT and [^{14}C]DDE and to the lowest concentration of [^{14}C]DDD used in the toxicity experiment (Table 2) in 1-L beakers filled with 800 ml of spiked Huron River water. After a 48-h static exposure, amphipods were transferred in equal numbers to 300-ml beakers containing 100 ml of uncontaminated Lake Michigan sediment and 200 ml of uncontaminated Huron River water. Three-

fourths of the water was exchanged twice weekly. Amphipods were retrieved from one beaker per treatment after 0, 2, 10, 30, 60, and 90 d, and these were individually weighed and assayed for radioactivity using LSC. Sediment was used as a substrate with *Diporeia* spp. to ensure survival during prolonged elimination periods.

Biotransformation

Hyallela azteca (n = 200) were exposed to [^{14}C]DDT (0.092 μg/L), [^{14}C]DDD (0.207 μg/L), or [^{14}C]DDE (1.682 μg/L) in 1-L beakers filled with 800 ml of spiked Huron River water. After a 24-h static exposure, amphipods from each beaker were retrieved and stored frozen at −20°C before analysis for biotransformation products. In addition, *H. azteca* were exposed to DDT at 0.092 μg/L for 10 d. Three-fourths of the test solution in each beaker was exchanged daily, and amphipods were fed 2 ml of YCT every second day. *Diporeia* spp. exposed to DDT at 0.221 μg/L, DDD at 0.944 μg/L, and DDE at 2.293 μg/L in the 28-d toxicity bioassay were sampled at test termination and stored frozen. Amphipods sampled from replicate beakers were pooled and stored at −20°C before analysis of biotransformation products.

Frozen organisms were thawed at room temperature and blotted dry on paper towels. Amphipods were sonicated for 20 s in 12 ml of ethylacetate:acetone (1:4, v:v). The extract was filtered through a sodium–sulfate column. The residual tissue was re-extracted twice with 12 ml of cyclohexane. All extracts were combined and the volume reduced to approximately 0.5 ml under a stream of nitrogen. The extracts were analyzed by thin-layer chromatography (TLC) on precoated silica gel 60F–254EM glass plates (Alltech, Deerfield, IL, USA) using hexane:benzene (95:5, v:v). Developed plates were divided into 1-cm sections starting from the origin. The silica gel was removed, and the [^{14}C] activity was determined using LSC. The locations on the plate of sections corresponding to DDT, DDD, and DDE standards were determined by visual observation under ultraviolet light. The relative fraction of DDT, DDD, DDE, or polar metabolites in the exposed organisms was calculated by dividing the radioactivity of selected sections by the radioactivity of all sections combined. For *H. azteca,* tissue samples were analyzed in triplicate; for *Diporeia* spp., tissue extractions were not replicated.

Modeling accumulation

Contaminant accumulation and loss kinetics were determined using a two-compartment model with water as the source and the organisms as the recipient. Because the water concentrations fluctuated and declined in some cases by as much as 61% during the period between daily water changes, assumptions of constant water concentrations would be invalid. Water samples were taken daily both before and after exchanging the water. Thus, the total loss was known, and the compound concentration was assumed to decline linearly between water renewals. Rate coefficients were then estimated by numeric integration using the differential form of the two-compartment model

$$\frac{dC_a}{dt} = k_u(C_w^{t=t_i} - m_i t) - k_e C_a$$

where k_u is the uptake clearance rate (ml g^{-1} h^{-1}), $C_w^{t=t_i}$ is the concentration in the water (nmol ml^{-1}), $t_i \le t \le t_{i+1}$ and i equals 1 . . . N where N counts the number of changes in slope because of water changes, m_i is the slope of the linear decline for the water concentration at each interval, C_a is the concentration in the organism (nmol g^{-1}), k_e is the elimination rate constant (h^{-1}), and t is time. Both k_u and k_e are conditional rates and depend on the experimental conditions for which they are measured, but they are assumed to be constant for that set of conditions. Initial estimates of k_u and k_e for numeric integration were made by fitting the data to the integrated form of the two-compartment model by using the time-weighted average water concentration as an assumed constant water concentration. The numeric integration was performed to provide a least-squares fit to the data using IMSL software (Visual Numerics, Houston, TX, USA) with the subroutine DIVPRK, which incorporates fifth- and sixth-order Runge-Kutta formulas.

Experimentally measured elimination rate constants ($k_{e(m)}$) for both species were estimated by fitting the data from the elimination experiments to a first-order decay [19]:

$$C_a^{t=t} = C_a^{t=0} e^{-ke(m)t}$$

where $C_a^{t=0}$ is the compound concentration in the amphipods at the beginning of the elimination experiment. The corresponding half-lives ($t_{½}$) were determined in terms of $k_{e(m)}$ by the formula

$$(t_{½}) = 0.693 k_{e(m)}^{-1}$$

Statistics

For the toxicity tests, one-way analysis of variance was used to analyze the amphipod survival data. Contaminant treatments were compared with the control treatment using the Williams' test. Significance level (α) was set at 0.05. Mortality data were transformed by arcsine-square-root before analysis. Median lethal concentration (LC50) or median lethal tissue residue (LR50) values were calculated using the trimmed Spearman-Karber method. Narcosis data were analyzed using the trimmed Spearman-Karber method for deriving the median effect concentration (EC50) or the median effect tissue residue (ER50). The relationship between percent survival and body residue was analyzed using a three-parameter, sigmoidal, nonlinear regression.

RESULTS

Water concentrations

Most of the [^{14}C] activity of DDT (97%), DDD (98%), and DDE (91%) was associated with the freely dissolved fraction of the compound in the exposure water. Concentrations in the water were not corrected to the freely dissolved fraction.

Contaminant concentration in the exposure water declined during the period between two consecutive partial renewals (1-d period) during the *H. azteca* and *Diporeia* spp. toxicity bioassays. For DDT, the mean decline in exposure concentration ranged from 25 to 39% across treatments in the *H. azteca* bioassay and from 33 to 43% in the *Diporeia* spp. bioassay. Mean decline in the water concentration of DDD was generally higher for *H. azteca* (33–41%) than for *Diporeia* spp. (27–34%). The highest mean decline was observed for DDE, ranging from 37 to 62% for *H. azteca* and from 41 to 46% for *Diporeia* spp.

Because the exchange of exposure water was generally performed at different times each day, the period between consecutive daily renewals varied from 18 to 38 h in all experiments. To account for this, time-averaged water concentrations were calculated as the exposure concentrations for the entire

Table 3. Median lethal concentration (LC50), median lethal residue (LR50), mean effect concentration (EC50), and mean effect tissue residue (ER50) calculated for *Hyalella azteca* in toxicity experiments with dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyltrichloroethylene (DDE)[a]

Point estimate	DDT	DDD	DDE
4-d LC50 (μg/L)	0.17 (0.17–0.18)	ND	10.99 (10.58–11.43)
10-d LC50 (μg/L)	0.10 (0.10–0.10)	0.77 (0.74–0.80)	3.88 (3.56–4.22)
4-d LR50 (μmol/g wet wt)	0.007 (0.007–0.008)	ND	0.999 (0.969–1.034)
10-d LR50 (μmol/g wet wt)	0.006[b] (0.006–0.007)	0.047 (0.041–0.051)	0.389 (0.364–0.416)[c]

[a] Numbers in parentheses indicate 95% confidence limits. ND = not determined.
[b] 10-d LR50 for DDT = 0.002 μmol/g wet wt when corrected for biotransformation products.
[c] Calculated using day 6 tissue concentration for 8.208 μg/L amphipods.

duration of the experiment for each treatment (Tables 1 and 2).

Hyalella azteca *mortality*

Mean ± SD control survival at termination of the experiment (day 10) was 92 ± 8.2% for DDT and 100 ± 0% for DDD and DDE. Mean percent survival of *H. azteca* declined with increasing contaminant concentration in the water (Table 1). Survival was significantly lower than in the control group at DDT concentrations of 0.122 μg/L or greater, DDD concentrations of 0.692 and 1.381 μg/L, and DDE concentrations of 2.258 μg/L and greater. For DDT and DDE, the 10-d LC50 was lower than the 4-d LC50 (Table 3). A 4-d LC50 could not be calculated for DDD because of low mortality. The 10-d LC50 value was lowest for DDT, followed by DDD then DDE, with the relative proportion among the values approximately 1:8:39.

Overall, a decline in survival was strongly associated with increasing body residues for all compounds (Fig. 1). At day 4, survival was assessed by nondestructive observation from replicate beakers, whereas body residues were measured from separate beakers by destructive sampling. Therefore, multiple survival data are associated with a single mean body residue measurement. At day 10, survival and body residue measurements were both obtained from each of four (five for DDT) replicate beakers. Because body residues were measured from live organisms only, an association could not be established between body residue and survival when complete mortality occurred. For DDT, body residues as low as 0.010 μmol/g wet weight were generally associated with low survival (<40%) at day 4. Survival correlated poorly with body residue at day 10 ($r^2 = 0.245$, $p = 0.07$), likely because only one treatment had significant partial mortality. Compared with DDT, survival declined at higher DDD body resides (0.4–0.18 μmol/g wet wt) (Fig. 1). Compared with DDT and DDD, much higher body residues of DDE were associated with a decrease in survival (Fig. 1). At day 4, survival was significantly lower at mean body residues of 0.725 μmol/g or greater. At day 10, low survival (40–80%) was associated with body residues in the range of 0.200 to 0.400 μmol/g wet weight.

To calculate a 10-d LR50 for DDE, body residue at day 6 was used as a surrogate for body residue at day 10 for the 8.202-μg/L treatment because of complete mortality at that treatment by day 10. For DDT and DDE, 10-d LR50 values were lower than 4-d LR50 values (Table 3). A 4-d LR50 could not be calculated for DDD because of low mortality. The 10-d LR50 values were lowest for DDT, followed by DDD then DDE, with the relative proportion among the values approximately 1:8:63.

Diporeia *spp. mortality*

Mean ± SD control survival at termination of the experiment (day 28) was 100 ± 0% for DDT, DDD, and DDE. Mean percent survival of *Diporeia* spp. declined, however, with increasing concentrations of DDT and DDD in the exposure water (Table 2). Survival was significantly lower than in the control group at DDT concentrations of 0.352 μg/L or greater and DDD concentrations of 0.944 μg/L or greater. For DDT and DDD, 28-d LC50 values were lower than 10-d LC50 values, and 28-d LC50 values for DDT were lower than those for DDT (Table 3), with the relative proportion among the values approximately 1:8. Within the range of concentrations used in this experiment, DDE was not toxic to *Diporeia* spp. Mean survival at the highest DDE treatment was 90% (Table 2). The mean measured water concentration for this treatment was 75-fold higher than the 28-d LC50 value for DDT and 10-fold higher than the 28-d LC50 value for DDD. This mean concentration (20.194 μg/L) was likely approaching the water solubility limit for DDE in water at 4°C [20].

Overall, *Diporeia* spp. survival decreased with increasing body residues of DDT and DDD, but survival remained high with DDE (Fig. 2). Mean DDT body residues as low as 0.045 mol/g wet weight resulted in a significant decrease in survival at day 28. Compared with DDT, decreased survival only occurred at much higher DDD body residues (0.2–0.5 μmol/g wet wt) (Fig. 2). At day 10, mean percent survival was greater than 70% at a mean body residue of 0.308 μmol/g wet weight and declined to 18% at a mean body residue of 0.421 μmol/g wet weight. At day 28, low survival (<80%) was associated with body residues as low as 0.195 μmol/g wet weight. Compared with those of DDT and DDD, much higher body residues of DDE were attained during the 28-d water exposure (1.5 μmol/g wet wt) (Fig. 2). Survival, however, remained high and did not decrease with increasing tissue concentrations.

For DDT and DDD, 28-d LR50 values were lower than 10-d LR50 values, and 28-d LR50 values for DDT were lower than those for DDD (Table 4), with the relative proportion between the 28-d LR50 for DDT and DDD approximately 1:6. A 10- or 28-d LR50 was not calculated for DDE because of low mortality. The highest DDE mean body residue measured at day 28 (1.352 μmol/g wet wt) was associated with a mean survival of 90%. This body residue was 31-fold higher than the 28-d LR50 value for DDT and fivefold higher than the 28-d LR50 value for DDD.

Narcosis

The number of live *Diporeia* spp. that were active (i.e., nonnarcotized) in each exposure beaker was recorded at days

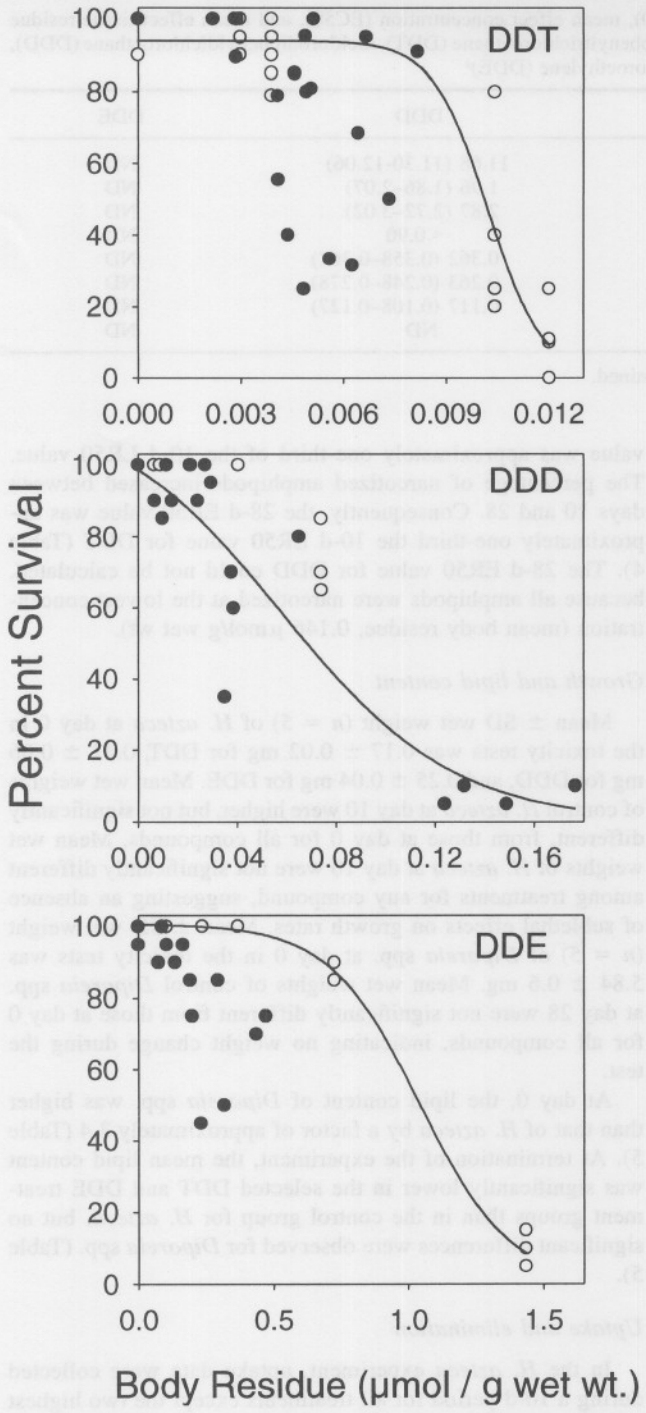

Fig. 1. *Hyalella azteca* toxicity experiments with DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE) examining percent survival versus body residues (day 10) or mean body residues (day 4). *Empty circles* represent day 4 data, and *filled circles* represent day 10 data. *Solid lines* represent the best fit from a three-parameter, sigmoidal, nonlinear regression for day 4 (DDT and DDE) or day 10 (DDD). For DDT, $r^2 = 0.905$ and $p < 0.001$. For DDD, $r^2 = 0.845$ and $p < 0.001$. For DDE, $r^2 = 0.996$ and $p < 0.001$.

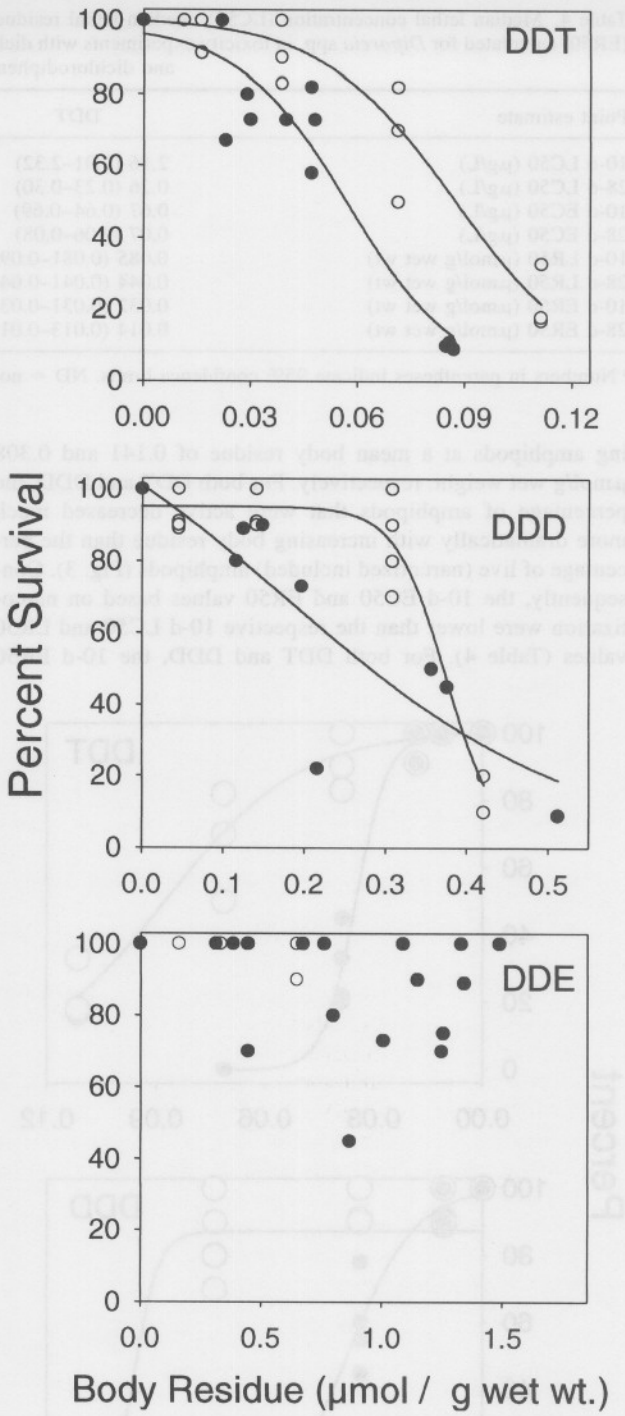

Fig. 2. *Diporeia* spp. toxicity experiments with DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE) examining percent survival versus body residues (day 28) or mean body residues (day 10). *Empty circles* represent day 10 data, and *filled circles* represent day 28 data. *Solid lines* represent the best fit from a three-parameter, sigmoidal, nonlinear regression for day 10 or day 28. For DDT, $r^2 = 0.944$ and $p < 0.001$ at day 10, and $r^2 = 0.921$ and $p < 0.001$ at day 28. For DDD, $r^2 = 0.795$ and $p < 0.001$ at day 10, and $r^2 = 0.959$ and $p < 0.001$ at day 28.

10 and 28 of the DDT, DDD, and DDE toxicity tests. Active amphipods were those able to actively swim on contact with a probe. The relationship between mean body residue and the percentage of amphipods either alive or alive and active (narcotized amphipods excluded) at day 10 in the DDT and DDD toxicity test is compared in Figure 3. For the DDT experiment, narcotized organisms comprised 62% and 100% of all surviving amphipods at a mean body residue of 0.039 and 0.071 µmol/g wet weight, respectively. For the DDD experiment, narcotized organisms comprised 37% and 100% of all surviv-

Table 4. Median lethal concentration (LC50), median lethal residue (LR50), mean effect concentration (EC50), and mean effect tissue residue (ER50) calculated for *Diporeia* spp. in toxicity experiments with dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE)[a]

Point estimate	DDT	DDD	DDE
10-d LC50 (μg/L)	2.16 (2.01–2.32)	11.68 (11.30-12.06)	ND
28-d LC50 (μg/L)	0.26 (0.23–0.30)	1.96 (1.86–2.07)	ND
10-d EC50 (μg/L)	0.67 (0.64–0.69)	2.87 (2.72–3.02)	ND
28-d EC50 (μg/L)	0.07 (0.06–0.08)	<0.90	ND
10-d LR50 (μmol/g wet wt)	0.085 (0.081–0.090)	0.362 (0.358–0.367)	ND
28-d LR50 (μmol/g wet wt)	0.044 (0.041–0.048)	0.263 (0.248–0.278)	ND
10-d ER50 (μmol/g wet wt)	0.032 (0.031–0.033)	0.117 (0.108–0.127)	ND
28-d ER50 (μmol/g wet wt)	0.014 (0.013–0.016)	ND	ND

[a] Numbers in parentheses indicate 95% confidence limits. ND = not determined.

ing amphipods at a mean body residue of 0.141 and 0.308 μmol/g wet weight, respectively. For both DDT and DDD, the percentage of amphipods that were active decreased much more dramatically with increasing body residue than the percentage of live (narcotized included) amphipods (Fig. 3). Consequently, the 10-d EC50 and ER50 values based on narcotization were lower than the respective 10-d LC50 and LR50 values (Table 4). For both DDT and DDD, the 10-d ER50 value was approximately one-third of the 10-d LR50 value. The percentage of narcotized amphipods increased between days 10 and 28. Consequently, the 28-d ER50 value was approximately one-third the 10-d ER50 value for DDT (Table 4). The 28-d ER50 value for DDD could not be calculated, because all amphipods were narcotized at the lowest concentration (mean body residue, 0.146 μmol/g wet wt).

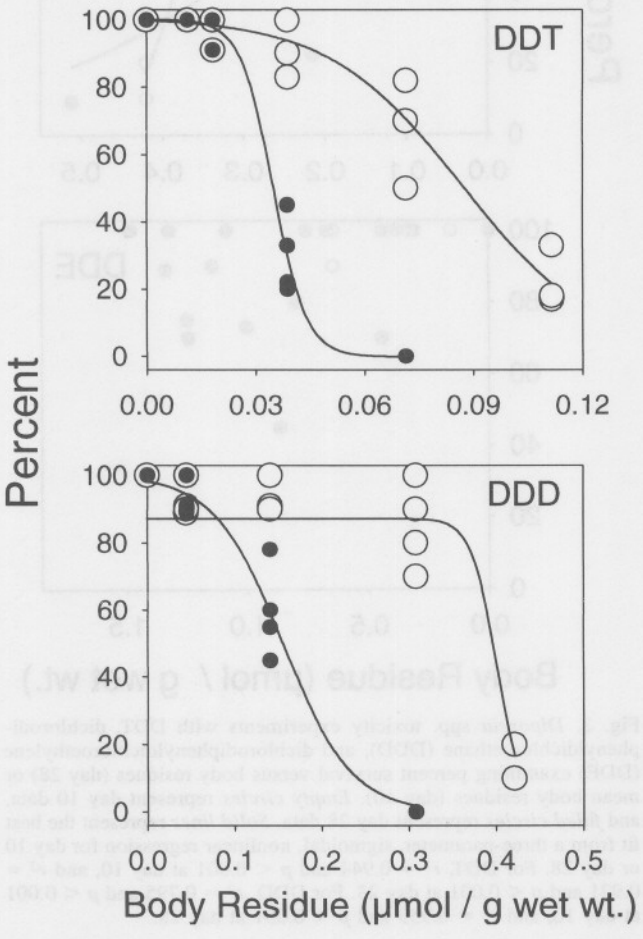

Fig. 3. *Diporeia* spp. toxicity experiments with DDT and dichlorodiphenyldichloroethane (DDD) examining percent survival (*empty circles*) or percent nonnarcotized (*filled circles*) versus mean body residues after a 10-d exposure. *Solid lines* represent the best fit from a three-parameter, sigmoidal, nonlinear regression. For DDT, $r^2 = 0.945$ and $p < 0.001$ for percent survival, and $r^2 = 0.987$ and $p < 0.001$ for percent active. For DDD, $r^2 = 0.909$ and $p < 0.001$ for percent survival, and $r^2 = 0.972$ and $p < 0.001$ for percent active.

Growth and lipid content

Mean ± SD wet weight ($n = 5$) of *H. azteca* at day 0 in the toxicity tests was 0.17 ± 0.02 mg for DDT, 0.41 ± 0.06 mg for DDD, and 0.25 ± 0.04 mg for DDE. Mean wet weights of control *H. azteca* at day 10 were higher, but not significantly different, from those at day 0 for all compounds. Mean wet weights of *H. azteca* at day 10 were not significantly different among treatments for any compound, suggesting an absence of sublethal effects on growth rates. Mean ± SD wet weight ($n = 5$) of *Diporeia* spp. at day 0 in the toxicity tests was 5.84 ± 0.6 mg. Mean wet weights of control *Diporeia* spp. at day 28 were not significantly different from those at day 0 for all compounds, indicating no weight change during the test.

At day 0, the lipid content of *Diporeia* spp. was higher than that of *H. azteca* by a factor of approximately 3.4 (Table 5). At termination of the experiment, the mean lipid content was significantly lower in the selected DDT and DDE treatment groups than in the control group for *H. azteca,* but no significant differences were observed for *Diporeia* spp. (Table 5).

Uptake and elimination

In the *H. azteca* experiment, uptake data were collected during a 10-d period for all treatments except the two highest DDT and DDE concentrations, in which complete mortality occurred before the termination of the experiment. The uptake rate constants (k_u) for DDT, DDD, and DDE were lowest at high exposure concentrations, in which survival was significantly impacted (Table 1). No apparent relationship was evident between exposure concentration and elimination rate (k_e) for DDT and DDD (Table 1). For DDE, estimates of k_e at concentrations of 8.208 and 22.021 μg/L were exceedingly low (Table 1), likely because of the linear nature of the uptake curve, which was derived from data collected for only 4 days because of complete mortality. Considering only treatments in which survival was not significantly impacted, k_u and k_e values were similar for DDT and DDD and higher for DDE.

In the *Diporeia* spp. experiment, uptake data were collected during a 28-d period for all treatments except 1.595 and 3.319

Table 5. Mean (standard deviation) total lipid content in *Hyalella azteca* or *Diporeia* spp. sampled at the initiation (day 0) or termination (day 10 or 28) from selected treatments in the toxicity experiments

Compound[a]	Sample	Percentage of lipids, mean (SD)
Hyalella azteca		
DDT	day 0	7.2 (0.8)
DDT	day 10, control	7.7 (1.5)
DDT	day 10, 0.122 μg/L	4.6 (0.6)[b]
DDD	day 0	ND
DDD	day 10, control	7.0 (1.1)
DDD	day 10, 0.682 μg/L	6.5 (0.6)
DDE	day 0	6.9 (0.9)
DDE	day 10, control	7.5 (0.9)
DDE	day 10, 4.947 μg/L	5.0 (2.3)[b]
Diporeia spp.		
DDT, DDD, DDE	day 0	23.7 (8.5)
DDT, DDD, DDE	day 28, control	23.9 (6.3)
DDT	day 28, 0.352 μg/L	22.2 (4.4)
DDD	day 28, 0.944 μg/L	24.3 93.4)
DDE	day 28, 20.194 μg/L	26.7 (5.1)

[a] DDT = dichlorodiphenyltrichloroethane; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.
[b] Denotes samples significantly different from controls.

μg/L for DDT and 7.420 and 17.050 μg/L for DDD, in which complete mortality occurred before termination of the experiment. For DDT, k_u values were within a narrow range across concentrations (Table 2). For DDD, k_u was highest at 7.420 and 17.050 μg/L, in which complete mortality occurred between days 10 and 17 (Table 2). The uptake rate constant (k_u) for DDE declined significantly with increasing compound concentration in the water, but survival was not impacted in any treatment (Table 2). Overall, k_u values for DDT, DDD, and DDE did not differ greatly, and the range for each compound overlapped. The k_e values estimated for DDT were highest for those treatments in which full mortality occurred before the end of the 28-d exposure (1.595 and 3.319 μg/L, Table 2). A similar but more striking pattern was observed with DDD, for which the k_e values were exceedingly high at 7.420 and 17.050 μg/L. The highest k_e value for DDE was observed at 20.194 μg/L, which was the highest concentration tested. The k_e values determined for the two lowest concentrations of DDT, DDD, and DDE were very similar.

Comparing only treatments in which survival was high (>80%), the k_u values determined for *H. azteca* were greater than those for *Diporeia* spp. by factors of approximately four to six for the three compounds studied. Likewise, k_e values for *H. azteca* were higher those for *Diporeia* spp. by factors of approximately 10 to 20.

Experimentally measured elimination

In the *H. azteca* elimination experiment, estimates of $k_{e(m)}$ for DDT were similar across treatments and were in the same range as the estimates of k_e (Table 1). The $k_{e(m)}$ values for DDD and DDE were somewhat higher than that for DDT and similar to the corresponding k_e estimates. Elimination half-lives ($t_{½}$) ranged from 2.8 to 3.9 d (68–94 h) for DDT and were 1.5 and 2.3 d (35 and 56 h) for the selected DDD and DDE treatments, respectively.

In the *Diporeia* spp. elimination experiment, all estimates of $k_{e(m)}$ were identical except for the DDT exposure of 0.221 μg/L (Table 2). Compared with k_e values, $k_{e(m)}$ values for a given treatment were always lower. Differences as large as a factor of 10 were observed for the highest concentrations of DDT and DDE. The elimination half-life ($t_{½}$) was 144.4 d (3,465 h) for all treatments used in the elimination study except the DDT concentration of 0.221 μg/L, in which it was 96.3 d (2,310 h). Overall, compound elimination was much slower in *Diporeia* spp. than in *H. azteca.*

Steady state

The estimated steady-state bioconcentration factor (BCF_{ss}), which is calculated using the formula $k_u/k_{e(m)}$, was calculated only for the treatments used in the elimination experiment. For *H. azteca,* the BCF_{ss} values for DDT were similar across concentrations and showed no apparent correlation with the DDT water concentration (Table 1). The BCF_{ss} values were also similar among contaminants for each species, but BCF_{ss} values for *Diporeia* spp. were higher than those for *H. azteca* by a factor of approximately 10.

Visual inspection of uptake curves for *H. azteca* (not presented) indicated that amphipods were either at or close to attaining an apparent steady state at termination of the experiment. The exposure time in the *H. azteca* experiments (10 d) corresponded to at least three elimination half-lives ($0.693/k_{e(m)}$). Time to achieve 95% steady-state residues, which was calculated using the formula $2.99/k_{e(m)}$, was 12.2 to 16.8 d for DDT, 6.23 d for DDD, and 10.13 d for DDE. For *Diporeia* spp., visual inspection of uptake curves (not presented) indicated that tissue concentration was increasing almost linearly toward the end of the 28-d exposure time in most treatments. The 28-d exposure period corresponded to approximately one-third of one elimination half-life, further confirming that the test organisms were far from attaining steady state by the last sampling period. Time to achieve 95% steady-state residues, which was calculated using the formula $2.99/k_{e(m)}$, was 415 and 623 d for DDT and 623 d for both DDD and DDE.

The expected tissue concentration at steady state can be estimated using the time-averaged exposure water concentration and BCF_{ss}. For *H. azteca,* mean measured tissue concentrations at day 10 were similar or exceeded the expected tissue concentrations at steady state for all treatments in which $k_{e(m)}$ was determined except at 0.358 μg/L for DDT and 1.117 μg/L for DDE. For the latter two treatments, the mean measured concentration at day 10 corresponded to only 42 and 72%, respectively, of the modeled tissue concentrations at steady state. For *Diporeia* spp., mean tissue concentrations at day 28 corresponded to between 10 and 19% of the expected tissue concentration at steady state. The fraction of the steady-state tissue residue achieved at termination of the experiment can also be calculated with $k_{e(m)}$ using the equation $1 - e^{-k_{e(m)} \cdot t}$. This fraction was approximately 88% or greater for *H. azteca* but only approximately 13% for *Diporeia* spp.

Biotransformation

After a 24-h exposure to DDT at 0.092 μg/L, the mean ± SD fraction of [^{14}C] activity in *H. azteca* tissues corresponding to DDT was 75.0 ± 2.3%, and the fractions corresponding to DDE and polar metabolites were 23.6 ± 2.4% and 1.4 ± 0.3%, respectively. After a 10-d exposure to DDT the same water concentration, the fraction of DDT in *H. azteca* tissues was 34.4 ± 3.0%, and the fractions corresponding to DDE and polar metabolites were 64.4 ± 2.7% and 1.2 ± 0.4%, respectively. Dichlorodiphenyldichloroethane was not detected in either the 24-h or the 10-d exposure. After a 24-h exposure to

DDD at 0.207 μg/L or DDE at 1.682 μg/L, the fraction of [^{14}C] activity in *H. azteca* tissues corresponding to the parent compound was greater than 98%.

After a 28-d exposure to DDT at 0.221 μg/L, the fraction of DDT in *Diporeia* spp. tissues was 95.7%, and the fractions corresponding to DDD and polar metabolites were 4.0% and 0.3%, respectively. Dichlorodiphenyldichloroethylene was not detected. After a 28-d exposure to DDD at 0.944 μg/L or to DDE at 2.293 μg/L, the fraction of the parent compound was greater than 98%.

DISCUSSION

Exposure concentrations and toxicity

In terms of compound water concentrations, DDT was more toxic to *H. azteca* than either of its metabolites, followed by DDD then DDE, as previously determined by Hoke et al. [3]. Our 10-d LC50 estimates for *H. azteca* (0.10, 0.77, and 3.88 μg/L for DDT, DDD, and DDE, respectively) were similar to those obtained by Hoke et al. (0.07, 0.19, 1.66 μg/L, respectively). That the exposures in Hoke et al. were flowthrough might have accounted for some of the higher observed toxicity in their study. In 10-d static exposures to DDT, Nebeker et al. [21] calculated LC50 values that ranged from 0.46 to 0.48 μg/L. That Nebeker et al. employed 2-month-old amphipods suggests that sensitivity may decrease with age. As noted by Phipps et al. [22], *H. azteca* ranks as the most sensitive aquatic invertebrate to DDT. *Diporeia* spp. was also more sensitive to DDT than to DDD or DDE in our study; no significant decrease in survival was observed in the 28-d exposure to DDE.

When comparing 10-d LC50 values for *H. azteca* and *Diporeia* spp., values for *Diporeia* spp. were higher by a factor of 22 for DDT and by a factor of 15 for DDD. When comparing 10-d LC50 values for *H. azteca* with 28-d LC50 values for *Diporeia* spp., the sensitivity difference between the two species was drastically diminished. Because *H. azteca* was close to attaining steady-state tissue concentrations at day 10, the LC50 measured at day 10 should be similar to the incipient LC50 for DDT, DDD, and DDE. Conversely, *Diporeia* spp. exposed to the same compounds were far from attaining a steady state even at the end of the 28-d exposure. The steady-state LC50 ($LC50_{ss}$) for *Diporeia* spp. exposed to DDT can be estimated by dividing the lethal critical body residue (CBR) by the BCF_{ss} [23]. For DDT, using the 28-d LR50 as the lethal CBR and the BCF_{ss} for 0.221 μg/L, the estimated $LC50_{ss}$ (0.07 μg/L) was similar to the 10-d LC50 for *H. azteca* (0.10 μg/L). For DDD, using the 28-d LR50 as the lethal CBR and the BCF_{ss} for 0.944 μg/L, the estimated $LC50_{ss}$ (0.192 μg/L) was lower than the 10-d LC50 for *H. azteca* (0.77 μg/L). From an environmental perspective, *H. azteca* should be more susceptible than *Diporeia* spp. to short-duration, long-interval, pulsed exposures because of its relatively faster toxicokinetics. As the exposures become more constant and of longer duration, however, both organisms are similarly sensitive to these compounds.

Tissue concentrations and toxicity

The dose–response relationship for DDT was established using [^{14}C] activity in the tissues as a surrogate for the DDT concentration. Analysis of the identity of contaminants in *H. azteca* exposed to a water concentration (0.092 μg/L) similar to the 10-d LC50 for DDT revealed that DDT accounted for only 34% of the total [^{14}C] activity, whereas DDE accounted for 64%. Applying these fractions, the fraction of the LR50 (0.006 μmol/g wet wt) corresponding to DDT was 0.006 • 0.34, or 0.0020 μmol/ g wet weight, and that of DDE was 0.006 • 0.64, or 0.0038 μmol/g wet weight. The number of DDE toxic units, which is calculated as the DDE tissue concentration divided by the 10-d LR50 for DDE, was negligible (0.01). It is fair to assume that mortality resulted entirely from the presence of DDT in the tissues of *H. azteca.* Therefore, our best estimate of the LR50 for DDT is 0.002 μmol/g wet weight. In *H. azteca,* DDT was more toxic than DDD by a factor of 24 and more toxic than DDE by a factor of 195. These ratios were considerably higher than the ratios between the corresponding LC50 values (8 and 39, respectively).

Diporeia spp. were substantially more tolerant than *H. azteca* to DDT and its metabolites. The 28-d LR50 values for *Diporeia* spp. were higher than the 10-d LR50 values for *H. azteca* by factors of 22 and six for DDT and DDD, respectively. Dichlorodiphenyldichloroethylene was not acutely toxic to *Diporeia* spp. even though the highest body residue measured at day 28 were greater than the *H. azteca* 10-d LR50 by a factor of four. These differences are partially explained by the higher total lipid content of *Diporeia* spp., which was approximately 24% of dry weight. at day 0, compared with that in *H. azteca*, which was approximately 7% at day 0. In both intra- and interspecific comparisons, organisms with a higher lipid content were typically more tolerant to a given contaminant [24–26]. Even after lipid normalization, lethal CBRs were still considerably higher for *Diporeia* spp. (28-d LR50 of 0.682 and 4.07 μmol/g lipid for DDT and DDD, respectively) than for *H. azteca* (10-d LR50 of 0.104 and 2.53 μmol/g lipid for DDT and DDD, respectively).

Direct observation of test organisms revealed that *Diporeia* spp. exposed to DDT and DDD became sluggish and increasingly immobilized long before death occurred. Body residues and exposure concentrations associated with narcosis were remarkably lower than those associated with mortality. Under constant exposure, narcotized animals, though not physiologically dead, can be considered to be ecologically dead, because they likely do not feed, reproduce, or escape from predators. The use of narcosis as a toxicity endpoint, though difficult or even impossible at times (e.g., sediment exposures), is more likely to provide ecologically relevant toxicity data than sole use of the mortality endpoint.

Striking differences in the CBRs for DDT, DDD, and DDE suggest marked differences in their mode of action. For nonpolar compounds acting solely by general narcosis, such as PAHs, LC50 values for different congeners span several orders of magnitude in aquatic organisms. Yet, lethal tissue concentrations for the same compounds expressed on a molar basis fall within a very close range [11]. Rather than acting solely as a general narcotic, DDT also exerts toxicity by more specific modes of action. It binds to the axonic membrane of the nerve fiber and impairs the normal functioning of voltage-sensitive sodium channels [27]. The onset of death from bioaccumulation of DDT is likely to result from this effect. The lethal CBR for DDT in our study was lower than the typical lethal CBR for narcotizing compounds in amphipods (2–6 μmol/g wet wt [28,29]) by a factor of approximately 1,000 in *H. azteca* and of approximately 50 in *Diporeia* spp. Low CBRs for DDD in *H. azteca* and *Diporeia* spp. also indicate toxicity by specific modes of action. Body residues of DDE as great as 1.46 μmol/g wet weight in *Diporeia* spp., however, were not associated

with significant lethality. It is likely that DDE elicits mortality in *Diporeia* spp. mostly through general narcosis. For *H. azteca,* the 10-d LR50 for DDE (0.39 nmol/g wet wt) was lower than the estimated 10-d LR50 for the general narcotic fluoranthene (3.6 and 5.6 μmol/g wet wt [28]), suggesting that modes of action other than general narcosis are operative but not as strongly as with DDT or DDD. The biotransformation of DDT to DDE acts as a mechanism of detoxification and is protective to *H. azteca* in terms of lethality. Biotransformation of DDT has the opposite effect in some species of freshwater planarians, however, because the metabolites DDD and DDE are more toxic to these species than the parent compound DDT [9].

Toxicokinetics

A decline in water concentration was observed between water exchanges in the toxicity experiments for all compounds and likely resulted from accumulation by the organisms, sorption to glass or other materials, and volatilization. The accumulation of contaminants in small aquatic organisms has generally been modeled using simple kinetics models that assume fixed concentrations for exposure and treat the organism as a single compartment [19]. Because this assumption was violated in our toxicity experiments, our modeling approach accounted for the changing water concentration by assuming the concentration declined linearly between each exchange. The decline in water concentration can also, however, be assumed to be first order, in which the decline is proportional to the water concentration. In the highest decline observed during this study (62%), time-averaged water concentration available for exposure, or the forcing function, was overestimated by 10% by assuming a linear decline compared with assuming a first-order decline. Because intermediate measurements were not made, it is not possible to determine which model most accurately reflects the actual decline.

For *H. azteca,* the uptake clearance rate (k_u) for DDD and DDE was relatively lower among treatments in which the mortality was high, suggesting a potential impact of toxicity on kinetic estimates. It has been previously reported that aquatic animals accumulate organic contaminants less efficiently when they are sick or when they accumulate deleterious doses of hydrophobic contaminants [28,30]. An inverse trend was observed with DDD for *Diporeia* spp., however, in which the most efficient uptake occurred among treatments in which all test organisms died during the 28-d exposure. A positive impact of toxicity on uptake rates has also been observed in *Diporeia* spp. exposed to sediments spiked with PAHs [29]. With DDT, no impact of toxicity on k_u was apparent for *H. azteca* or *Diporeia* spp.

A comparison of the uptake clearance rates for DDT, DDD, and DDE measured at the lowest water concentration, in which the organisms are least intoxicated, reveals that k_u was highest for DDE and lowest for DDT in both *H. azteca* and *Diporeia* spp. Uptake rates are expected to increase with increasing log K_{ow} and to decrease with increasing water solubility [31]. Of the three compounds used in this study, DDD is the least hydrophobic (log K_{ow} of 6.91, 6.22, and 6.96 for DDT, DDD, and DDE, respectively [32]). Therefore, it was surprising that DDD appeared to be taken up more efficiently than DDT by both amphipod species.

The range of k_u values measured for DDT, DDD, and DDE were higher in *H. azteca* than in *Diporeia* spp. by a factor of from three to five. The *H. azteca* used in this study were smaller than the *Diporeia* spp., and rates of uptake vary with organism size [19]. Smaller organisms typically have larger surface area–to–volume ratios, which lead to higher contaminant influx via the integument compared with that in larger organisms. The much higher water temperature in the *H. azteca* experiments likely further contributed to higher uptake rates. Nawaz and Kirk [33] reported that *Daphnia pulex* bioaccumulated increasingly more DDE over a fixed period of time with increasing temperature, and Landrum [31] reported that uptake of highly hydrophobic PAHs and PCBs by *Diporeia* spp. increased with exposure temperature. In our study, *H. azteca* were fed YCT during the exposure, whereas *Diporeia* spp. were not fed. Dichlorodiphenyltrichloroethane and its metabolites are expected to strongly sorb to food particles. That *H. azteca* therefore had a compartment source for compound uptake other than the exposure water might also have contributed to the faster uptake rate. To our knowledge, few studies have examined the bioaccumulation kinetics of DDT and its metabolites in aquatic invertebrates, but the DDT uptake rate in *H. azteca* was in the same range as that measured in euphausiids and copepods [30]. In addition, the mean uptake rate of DDE in *Diporeia* spp. obtained in this study fell well within the range of k_u values previously determined at trace concentrations by Evans and Landrum [34].

For *H. azteca,* there was no apparent relationship between elimination rate (k_e) and compound concentration in the water except for the two highest DDE concentrations, in which complete mortality by day 7 prevented an accurate calculation of the elimination rate from the uptake curve. For *Diporeia* spp., however, k_e tended to increase with increasing water concentration for all compounds and more strongly for DDD. Intoxicated *Diporeia* spp. may eliminate DDT and its metabolites at a faster rate. Alternatively, the rate of compound uptake may decrease as organisms become more intoxicated, as speculated when *H. azteca* and *Diporeia* spp. were exposed to fluoranthene [28]. Because the nonlinear model used to estimate k_e assumes that k_u is constant throughout the experiment, a decrease in k_u with time would result in a slower increase in tissue concentrations toward the end of the experiment, which forces the uptake curve toward an asymptote and results in an inaccurately high estimate of k_e. For *H. azteca,* experimentally measured elimination rate coefficients ($k_{e(m)}$) were similar to the estimated k_e values. For *Diporeia* spp., $k_{e(m)}$ values were similar to k_e values at the lowest concentrations of all compounds, but they differed greatly as the highest concentrations of DDT and DDD, likely because of the effects of intoxication on contaminant uptake discussed earlier.

For both *H. azteca* and *Diporeia* spp., estimates for k_e were similar among the three compounds investigated. It should be noted, however, that the model used to derive k_e is limited to describing the kinetics for the total radioactivity. For *Diporeia* spp., modeled kinetics reflects the parent compound in all cases because of the limited biotransformational capability. For *H. azteca,* the model kinetics reflects the parent compound for DDE and DDD for update, but for DDT the uptake is parent (DDT) but the elimination reflects a combination of biotransformation and elimination of the metabolite (DDE) and of the parent. In the case of DDT in *H. azteca,* insufficient data were collected to model the kinetics in more detail. An estimate of the rate constant for biotransformation and the elimination rate constant for parent DDT, however, can be calculated from the biotransformation data and the elimination data for DDE. The half-life for total DDT is in the range of 2.8 to 3.9 d; therefore,

by day 10, the *H. azteca* body residue will be between 82 and 91% of the steady-state value. Assuming a steady state, the following model represents the kinetics for both the parent compound (DDT) and the metabolite (primarily DDE):

$$0 = \frac{dC_p}{dt} = k_u C_w - k_m C_p - k_{ep} C_p \quad \text{and}$$

$$0 = \frac{dC_m}{dt} = k_m C_p - k_{em} C_m$$

where C_p is the concentration of parent compound in the organism, k_u is the measured uptake clearance for DDT, C_w is the concentration of DDT in the water, C_m is the concentration of metabolite in the organism, k_m is the rate constant for biotransformation, k_{ep} is the rate constant for elimination of the parent compound, k_{em} is the rate constant for elimination of the metabolite DDE, and t is time. If the data for the lowest concentrations of DDT (BCF_{SS} = 27,172) are used along with the lowest concentration for DDE to provide measured rate constants and the biotransformational data are taken from the 10-d, low-level exposure, then estimates of k_m and k_{ep} are 0.025 and 0.005 h^{-1}, respectively. Thus, most of the measured total elimination from the DDT kinetics results from elimination of the metabolite DDE, with only a small portion coming from DDT.

Estimates of k_e and $k_{e(m)}$ were much lower for *Diporeia* spp. than for *H. azteca,* especially at low water concentrations. Elimination half-lives were 1.4 and 3.9 d in *H. azteca* and from 96 to 144 d in *Diporeia* spp. Likely factors contributing to this difference are the much higher ambient temperature, lower lipid content, and the greater surface area–to–volume ratio for *H. azteca.* Also, based on the estimate of the elimination rate constant for DDT versus DDE in *H. azteca,* the conversion accelerates elimination of total compound.

Steady-state bioconcentration factors for *Diporeia* spp., which are calculated using the equation $k_u/k_{e(m)}$, were higher by a factor of at least eight compared with BCF_{ss} values calculated for *H. azteca.* The lipid content of *Diporeia* spp. at the beginning of the exposures was approximately threefold higher than that of *H. azteca,* only partly explaining the large difference observed between the two amphipod species. For *H. azteca,* the values of BCF_{ss} were higher for DDE, the most hydrophobic compound, and lower for DDD, the least hydrophobic compound. For *Diporeia* spp., the highest value was determined for DDE and the lowest for DDT.

Biotransformation

Diporeia spp. have a very limited ability to metabolize DDT, whereas *H. azteca* extensively biotransforms this compound. *Hyalella azteca* also biotransforms PAHs much more efficiently than *Diporeia* spp. [28,31,35]. The two species also differ in the metabolic pathway of DDT biotransformation. *Hyalella azteca* exposed to DDT used the dehydrochlorination pathway, leading to the formation of DDE. After a 10-d exposure to [^{14}C]-DDT, DDE comprised 64% of the total [^{14}C] activity in the tissues, whereas DDD was not detected. *Diporeia* spp. metabolized a small fraction of the accumulated DDT (4%) to DDD using the reductive dechlorination pathway. Formation of DDD has been observed in North American freshwater planarians [36,37], daphnids, and decapods [38]. Formation of DDE has been observed in a larger variety of animal species, including the amphipod *Gammarus fasciatus,* European planarians [10], midges, chironomids, dragonflies [38], and a few fish species [39,40].

SUMMARY AND CONCLUSIONS

Hyalella azteca exhibited more rapid kinetics than *Diporeia* spp. for the accumulation and loss of DDT, DDE, and DDD. Many of the differences in kinetics between the two species were attributed to the temperature, the organism size, and the respective lipid content. These differences in kinetics lead to predicted steady-state BCF values that will be 10-fold lower in *H. azteca* than in *Diporeia* spp. The ability of *H. azteca* to biotransform DDT to the less toxic DDE is protective for this species, and this biotransformational capability greatly exceeds that of *Diporeia* spp. The highest toxicity was exhibited by DDT, followed by DDD then DDE. For *H. azteca,* the body residue required to produce 50% mortality was estimated to be 0.002 μmol/g, which represented only approximately one-third of the overall body residue. Though contributing two-thirds of the body residue, DDE made little contribution to the toxicity. The finding of much lower toxicity for metabolites compared with DDT suggests that the relative proportion of DDT and its metabolites in field sites should be considered when assessing risk. The ΣDDT approach should only be used when comparing the toxicity of exposure matrices (e.g., sediments) with a similar proportion of DDT and its major metabolites. When the proportions vary significantly, this approach is not valid, because it assumes that all three compounds are equally toxic at any given concentration. When there is a disparity in the sensitivity of an organism to various compounds, the toxic-unit approach [4,41] should be favored over a simple summation approach for evaluating hazard.

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